学位の種類	博士（医学）
報告番号	甲第１８１６号
学位記番号	第１２９４号
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授与年月日	令和３年 ３月 24 日
学位論文の題名	AITC inhibits fibroblast-myofibroblast transition via TRPA1-independent MAPK and NRF2/HO-1 pathways and reverses corticosteroids insensitivity in human lung fibroblasts （ヒト肺線維芽細胞において、ワサビ辛味成分アリルイソチオシアネート（AITC）は、TRPA1 非依存的に ERK ならびに NRF2/HO-1 経路を介して線維芽細胞－筋線維芽細胞移行を抑制するとともに、副腎皮質ステロイド不応性を改善する） Respiratory Research (2021) 22:51. https://doi.org/10.1186/s12931-021-01636-9
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Abstract

There is limited knowledge on the role of transient receptor potential ankyrin 1 (TRPA1) in fibroblast - myofibroblast transition (FMT) that can lead to airway remodeling which is a major problem for severe asthma and fibrosis. Thus, this study investigated the effect of TRPA1 agonists and antagonists on transforming growth factor beta 1(TGF-β1) -treated lung fibroblasts.

This study utilized MRC-5 cells which were preincubated with TGF-β1 for 24 hrs. TRPA1 agonist or antagonist were added and further incubated for 24 hrs. The changes in TRPA1 and alpha-smooth muscle actin (α-SMA) expressions by stimuli were evaluated using qRT-PCR, western blot and immunohistochemical analyses. Statistical significance was determined by using one- or two-way ANOVA, followed by Bonferroni's post hoc analysis for comparison of multiple groups and paired 2-tailed Student's t-test between 2 groups.

Results showed that MRC-5 cells treated with TGF-β1 significantly upregulated α-SMA mRNA expressions ($P < 0.01$), but downregulated TRPA1 gene expression ($P < 0.001$). Post-treatment of TRPA1 activator, allyl isothiocyanate (AITC), after TGF-β1 significantly downregulated the α-SMA gene induction ($P < 0.01$ at 24h), protein expression ($P < 0.05$) and immunoreactivity with stress fibers ($P < 0.05$). On the other hand, TRPA1 antagonist HC-030031 did not prevent this effect, and instead tended to facilitate the suppressive effect of AITC when co-stimulated. AITC significantly increased phosphorylated- extracellular signal-regulated kinase (ERK) 1/2 and heme oxygenase (HO)-1 protein expressions ($P < 0.05$) in TGF-β1-treated cells. Combined inhibition with ERK1/2 mitogen-activated protein kinase (MAPK) and nuclear factor erythroid 2-related factor (NRF2) almost completely reversed AITC-induced α-SMA suppression ($P < 0.05$). Dexamethasone was not able to inhibit the upregulated α-SMA induction by TGF-β1. However, AITC improved dexamethasone-insensitive myodifferentiation in the presence of the corticosteroid ($P < 0.01$).

We reported for the first time that AITC exerts protective effect on TGF-β1-induced α-SMA induction by activating ERK1/2 MAPK and NRF2/HO-1 pathways in lung fibroblasts. It also overcomes corticosteroids insensitivity in TGF-β1-induced α-SMA induction. TRPA1 antagonist modulates the suppressive effect, but not prevent it. AITC and TRPA1 antagonist may be therapeutic agents in treating chronic respiratory diseases.